



Assessing Method Equivalency

6.1 Introduction

This chapter provides guidance on reviewing method validation study reports to assess whether a modified method has been demonstrated to produce results equivalent to results produced by the reference method. The guidance provided in this chapter is for use by regulatory authorities in assessing method equivalency when reference methods have been modified. Analytical laboratories may find the information in this chapter useful when validating a new or modified method.

According to streamlining procedures, validation study results for modified methods, regardless of tier, need not be submitted to EPA for approval. Rather, the organization responsible for developing the method modification must maintain on file complete records of all validation study documentation. Laboratories using the modification should provide a copy of the validation study report to all regulated entities whose samples are analyzed by the modified method. Regulated entities must retain validation study reports on file and make the files available for review on request by a regulatory authority or auditor.

Results of the method validations studies are documented on the *Checklist for Initial Demonstration of Method Performance*, the *Checklist for Continuing Demonstration of Method Performance*, and the *Certification Statement* (collectively called the “Checklists”). The Checklists are used by auditors and reviewers to evaluate new methods and method modifications against reference methods promulgated at Title 40 of the *Code of Federal Regulations* (CFR) parts 136 and 141. The process of assessing method equivalency involves (1) checking completeness of the method validation study report package, (2) reviewing the Checklists submitted in the validation package to ensure that the quality control (QC) acceptance criteria of the reference method have been met by the modified method, and (3) examining the raw data to clarify any questions or inconsistencies identified on the Checklists.

For Tier 1 method modifications, the completed Checklists, along with the raw data and example calculations, are adequate to document method equivalency, and a full method validation study report is not required. For all other validation tiers, the data reviewer must ensure that the validation study report is complete and includes all supporting data.

The key concepts presented and discussed in this chapter are: *the Checklists*, *completeness assessment*, *validation study report checksheet*, and *method equivalency assessment*.

6.2 Checking Completeness of the Method Validation Study Report Package

A method validation study report must be prepared for every study conducted to validate new or modified methods. Section 4.6 of this guide details the required contents of the method validation study report and the supporting data that must accompany the report. The following form can be used to check completeness of the validation package.

Table 6-1: Validation Study Report Checksheet

✓	Items Required
	Background section: Does it...
	Identify the method as a new method or a modification of a reference method?
	Include a method summary?
	If a modification, cite the organization and method number (given in 40 CFR parts 136, 141, and 405 - 503) for the reference method?
	If a modification, describe the reasons for and extent of the modification, the logic behind the technical approach to the modification, and the result of the modification?
	If a new method, describe the rationale for developing the method and explain how the method meets the criteria for a new method specified in the Streamlining Guide?
	Identify the matrices, matrix types, and/or media to which the method is believed to be applicable?
	List the analytes measured by the method or modification including corresponding CAS Registry or EMMI numbers? (Alternatively, is this information provided on the data reporting forms in the Supporting Data appendix to the validation study report? ____ Yes)
	Indicate whether any, some, or all known metabolites, decomposition products, or known commercial formulations containing the analyte are included in the measurement?
	State the purpose of the study?
	Study Design and Objectives section: Does it ...
	Describe the study design? [Validation study plan appended? ____ Yes]
	Identify overall objectives and data quality objectives of the study?
	Identify any study limitations?
	Study Implementation section: Does it ...
	Identify the organization that was responsible for managing the study?
	Identify the laboratories, facilities, and other organizations that participated in the study; describe how participating laboratories were selected; and explain the role of each organization involved in the study?
	Indicate at which Tier level the study was performed?

Table 6-1: Validation Study Report Checksheet

✓	Items Required
	Delineate the study schedule that was followed?
	Describe how sample matrices were chosen, including a statement of compliance with Tier requirements for matrix type selection?
	Explain how samples were collected and distributed?
	Specify the numbers and types of analyses performed by the participating laboratories?
	Describe how analyses were performed?
	Identify any problems encountered or deviations from the study plan and their resolution/impact on study performance and/or results?
	Data Reporting and Validation section: Does it ...
	Describe the procedures that were used and organizations involved in reporting and validating study data?
	Results section: Are results presented on the <i>Checklist for Initial Demonstration of Method Performance</i> , or in a tabular format attached to the Checklist?
	Are results presented on the <i>Checklist for Continuing Demonstration of Method Performance</i> , or in a tabular format attached to the Checklist?
	Is a signed <i>Certification Statement</i> attached to the Checklists?
	Development of QC Acceptance Criteria section (for new methods only):
	Does the section adequately describe the basis for development of QC acceptance criteria for all of the required QC tests?
	Data Analysis/Discussion section: Does it ...
	Provide a statistical analysis and discussion of the study results?
	For modified methods, address any discrepancies between the results and the QC acceptance criteria of the reference method?
	Conclusions section: Does it ...
	Describe the conclusions drawn from the study based on the data analysis discussion?
	Contain a statement(s) regarding achievement of the study objective(s)?
	Appendix A - The Method:
	Is it prepared in EPA format (i.e., in accordance with EPA's Guidelines and Format for Methods to be Proposed at 40 CFR Parts 136 or 141)?
	Appendix B - Validation Study Plan appended? (Optional)

Table 6-1: Validation Study Report Checksheet

✓	Items Required
	Appendix C - Supporting Data:
	Raw Data: Are raw data provided for all samples and QC analyses that will allow an independent reviewer to verify each determination and calculation performed by the laboratory by tracing the instrument output to the final result reported?
	Are the raw data organized so that an analytical chemist can clearly understand how the analyses were performed?
	Are the names, titles, addresses, and telephone numbers of the analysts who performed the analyses and of the quality assurance officer who will verify the analyses provided?
	Example Calculations: Are example calculations that will allow the data reviewer to determine how the laboratory used the raw data to arrive at the final results provided?

6.3 Assessing Equivalency Using the Checklists

The method validation results are reported on the Checklists. Copies of the Checklists and an example of completed Checklists are provided in Appendix E to this guide. The Checklists provide a side-by-side identification of the performance criteria (reference method QC acceptance criteria) and the results obtained in the validation study. A checkmark in the final column is used to indicate that the performance specifications of the reference method were achieved.

The data reviewer should review each item on the checklist to ensure that the QC acceptance criteria for each QC element were met. If there are any discrepancies, the reviewer should consult the data analysis/discussion section of the validation study report for a discussion of results and, if necessary, examine the raw data.

6.4 Data Review Guidance

This section provides guidance for reviewing data submitted to EPA and state authorities under CWA and SDWA. This guidance provides a tool for those who want to perform detailed inspection of data analyzed by methods under 40 CFR parts 136 and 141, to assess equivalency when method modifications are used or for other purposes. When performing equivalency assessments, any questions or discrepancies in the Checklists should be resolved by examining the raw data. The material presented in this section is technically detailed and is intended for data reviewers familiar with analytical methods.

6.4.1 Standardized Quality Control

In developing methods for the determination of pollutants and contaminants in water and in developing this streamlining initiative, EPA sought scientific and technical advice from many sources, including EPA's Science Advisory Board; scientists at EPA's environmental research laboratories;

scientists in industry and academia; scientists, managers, and legal staff at EPA Headquarters and Regions; States; contractors; contract laboratories; the regulated industry; consensus standards organizations; and others. The result of discussions held among these groups was the standardized quality control (QC) approach that is an integral part of the streamlined methods approval program. Standardized QC is specified for each reference method and contains the following elements:

- Calibration linearity
- Calibration verification
- Absolute and relative retention time precision (for chromatographic analyses)
- Initial precision and recovery or “start-up” tests
- Ongoing precision and recovery
- Analysis of blanks
- Surrogate or labeled compound recovery
- Matrix spike and matrix spike duplicate precision and recovery (for non-isotope dilution analyses)
- Demonstration of method detection limits
- Analysis of reference sample

When reviewing method validation data, the permit writer, PWS, or other individual or organization has the authority and responsibility to ensure that the test data submitted contain the elements listed above; otherwise, the data can be considered noncompliant.

6.4.2 Details of Data Review

The details of the data review process depend to a great extent upon the specific analytical method. Even for data from the same method, there may be many approaches to data review. However, given the standardized QC requirements of the streamlined methods approval program, a number of basic concepts apply. The following sections provide the details for reviewing analytical data and discuss EPA's rationale for the QC tests. Results from the QC tests for all standardized QC elements must be within the QC acceptance criteria specified in, or associated with, the reference method to validate that results produced by a method modification are equivalent or superior to results produced by the reference method.

6.3.2.1 Calibration linearity

The relationship between the response of an analytical instrument to the concentration or amount of an analyte introduced into the instrument typically is represented by an averaged response or calibration factor, a calibration line, or a calibration curve. An analytical instrument can be said to be calibrated in any instance in which an instrumental response can be related to a single concentration of an analyte. The response factor or calibration factor is the ratio of the response of the instrument to the concentration (or amount) of analyte introduced into the instrument.

Nearly all analytical methods focus on the range over which the response is a linear function of the concentration of the analyte. This range usually extends from the minimum level of quantitation (ML) on the low end to the point at which the calibration becomes non-linear on the high end. For regulatory compliance, it is important that the concentration of regulatory interest (e.g., permit limit; MCL) fall within this range. Calibration can also be modeled by quadratic or higher

order mathematical functions. The advantage of a calibration line that passes through the origin is that an averaged response factor or calibration factor can be used to represent the slope of this line. Use of a single factor simplifies calculations and the interpretation of the data. Also, it is easier to discern when an inaccurate calibration standard has been prepared if the calibration function is a straight line.

Many analytical methods, particularly recent methods, specify some criterion for determining the linearity of the calibration. When this criterion is met, the calibration function is sufficiently close to a straight line that passes through the origin to permit the laboratory to use an averaged response factor or calibration factor. Linearity is determined by calculating the relative standard deviation (RSD) of the response factor or calibration factor for each analyte and comparing this RSD to the limit specified in the method. If the RSD does not exceed the specification, linearity through the origin is assumed. If the specification is not met, a calibration curve must be used.

For whatever calibration range is used, a reference method should contain a specification for the RSD of the response or calibration factor to establish the breakpoint between linear calibration through the origin and a line not through the origin or a calibration curve. For new methods, the method developer must provide the RSD results by which one can judge linearity, even in instances where the laboratory is using a calibration curve. In instances where the laboratory employs a curve rather than an average response or calibration factor, the data reviewer should review each calibration point to ensure that the response increases as the concentration increases. If it does not, the instrument is not operating properly, or the calibration curve is out of the range of that instrument, and data are not considered valid.

6.3.2.2 Calibration verification

Calibration verification involves the analysis of a single standard at the beginning of each analytical shift or after the analysis of a fixed number of samples (e.g., 10). The concentration of each analyte in this standard is normally at the same level as in one of the calibration standards, typically at 1 - 5 times the ML. The concentration of each analyte in this standard is calculated using the calibration data. The calculated concentration is compared to the concentration of the standard. Calibration is verified when the concentration is within the calibration verification limits specified in the method. If the results are within the specifications, the laboratory is allowed to proceed with analysis without recalibrating and allowed to use the calibration data to quantify sample the concentration or amount of each analyte in samples, blanks, and QC tests.

If calibration cannot be verified, the laboratory may either recalibrate the instrument or prepare a fresh calibration standard and make a second attempt to verify calibration. If calibration cannot be verified with a fresh calibration standard, the instrument must be recalibrated. If calibration is not verified, subsequent data are considered to be invalid until the instrument is recalibrated.

6.3.2.3 Absolute and relative retention time precision

Retention time specification aid in the identification of analytes in chromatographic analyses. In some methods, a minimum retention time is specified to ensure adequate separation of analytes in complex mixtures. If retention time QC criteria cannot be verified, chromatographic identification of analytes is suspect and reanalysis is necessary.

6.3.2.4 Initial precision and recovery

This test is required prior to the use of the method by a laboratory. It is sometimes termed the "start-up test." Performing the start-up test "after the fact" or after samples have been analyzed is not acceptable. The laboratory must demonstrate that it can meet the IPR QC acceptance criteria in the method. EPA's experience has been that difficulty in passing the start-up test leads to marginal performance by the laboratory in the routine operation of the method.

The start-up test consists of spiking the analytes of interest into a set of four or more aliquots of a reference matrix and analyzing these four aliquots. The reference matrix simulates the medium being tested. A separate IPR test must be performed for each medium. The mean concentration and the standard deviation of the concentration are calculated for each analyte and compared to QC acceptance criteria in the method. If the mean and standard deviation are within the limits specified, the analysis system is in control and the laboratory can use the system for analysis of blanks, field samples, and other QC tests samples. For some methods (e.g., Methods 625 and 1625), a repeat test is allowed because of the large number of analytes being tested simultaneously.

If there are no start-up test data, or if these data fail to meet the QC acceptance criteria in the method, all data produced by that laboratory using that method are not considered valid. It is important to remember that if a change is made to a method, the start-up test must be repeated with the change as an integral part of the method. Such changes may involve alternative extraction, concentration, or cleanup processes; alternative GC columns, GC conditions, or detectors; or other procedures designed to address a particular matrix problem. If the start-up test is not repeated when a procedure is changed, added, or deleted, data produced by the modified method are considered invalid.

6.3.2.5 Ongoing precision and recovery

An ongoing precision and recovery (OPR) standard (also termed a "laboratory control sample" (LCS) or a "laboratory fortified blank" (LFB)) must be analyzed with each sample batch prior to the analysis of a blank, sample, or matrix spike or duplicate. The number of samples in the batch is usually 10 or 20, depending on the method, or the OPR is required at the beginning of an analysis shift, regardless of the number of samples analyzed during that shift. The data reviewer must determine if the OPR standard has been run with each sample batch or at the beginning of the shift and if all criteria have been met. If the standard was not run with a given set of samples, or if the criteria are not met, the results for that set of samples are considered invalid.

6.3.2.6 Analysis of blanks

Blanks must be analyzed either on a periodic basis or with each sample batch, depending on the method. Blanks may contain contamination at levels no higher than specified in the method. Samples associated with a contaminated blank must be reanalyzed.

6.3.2.7 Surrogate or labeled compound recovery

Surrogate or labeled compounds are used to assess the performance of the method on each sample. Recoveries of these compounds from each sample must be within QC acceptance criteria to demonstrate acceptable method performance on the sample. If the recovery is not within the criteria,

the sample is normally diluted and the dilute sample analyzed to demonstrate that a matrix effect precluded reliable analysis of the undiluted sample.

6.3.2.8 *Matrix spike and matrix spike duplicate*

Non-isotope dilution methods require a spike of the analytes of interest into a separate aliquot of the sample for analysis with the sample. The purpose of the matrix spike (sometimes termed a "laboratory fortified sample matrix" (LFM)) is to determine if the method is applicable to the sample in question. While many of the approved methods were tested using effluents from a wide variety of industries, samples from some sources may not yield acceptable results. It is therefore important to evaluate method performance in the sample matrix of interest. If the recovery for the MS/MSD is not within the QC acceptance criteria, a matrix interference may be the cause. The sample is usually diluted and the diluted sample spiked and analyzed. If the QC acceptance criteria are met with the diluted MS/MSD, a matrix problem exists. Cleanup and other processing of the sample are then required to overcome the matrix interference if analysis of the undiluted sample is required to establish compliance.

6.3.2.9 *Demonstration of method detection limits*

A laboratory that wishes to use a new or modified wastewater method must demonstrate that the method detection limit (MDL) specified in the reference method can be achieved. Alternatively, if the regulatory wastewater compliance limit is above the MDL, laboratories must demonstrate that the minimum level (ML) determined with the new or modified wastewater method is at or below 1/3 the compliance limit. A laboratory that wishes to use a new or modified drinking water method must demonstrate that the MDL determined with that method meets the detection limits specified at 40 CFR 141.23, 141.24, and 141.89 and/or as published in the table of QC limits in *Methods and Criteria*. For both drinking and waste water determinations, demonstration of a valid detection limit requires use of an MDL study in accordance with the procedure at 40 CFR part 136, Appendix B. If the MDL determined with the new or modified method is not acceptable, the method may not be used because the laboratory has not demonstrated an ability to detect the analyte at the level required. EPA notes that the required detection limits specified in the regulations and/or in the reference method(s) are usually analyte-specific; and for the same analyte the requirement may differ between the wastewater and the drinking water reference method.

6.3.2.10 *Reference Sample Analysis*

EPA is considering setting acceptance criteria for a reference material based on the measurement error of the method. Ideally, a laboratory should be able to demonstrate the ability to quantitate the analyte in a reference material to within the acceptance range specified for the reference material.